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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

APR 17 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: EPA ID # 084001: DDVP - Review of Metabolism of DDVP in Rats (MRID # 418399-01)

Tox. Chem. No: 328
Project No: 1-1556
Record No: S398178

From: Paul Chin, PhD 3/25/92
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Insecticide and Rodenticide Support (IRS)
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To: Larry Schnaubelt/Brigid Lowery, PM 72
Special Review and Reregistration Division (H7508W)

Thru: Joycelyn Stewart, Ph.D. 3/27/92
Acting Section Head
Section 2, Toxicology Branch I
Health Effects Division (H7509C) 4/11/92

I. CONCLUSIONS:

The Toxicology Branch I has reviewed the metabolism study for DDVP in rats. Data evaluation records are attached.

The Toxicology Branch I concludes that this study is acceptable, but it does not by itself satisfy the guideline requirements (85-1). In conjunction with a previous rat metabolism study (MRID No. 412287-01, HED DOC. NO. 008132 dated Oct. 19, 1990) these studies satisfy the guideline requirement for metabolism study.

II. ACTION REQUESTED:

Review and evaluate the following study:
Supplement to Metabolism of ^{14}C-DDVP in rats (preliminary and definitive phases). Study No. HLA 6274-105-1, MRID No. 418399-01, 3/28/91.

III. SUMMARY OF THE EVALUATION OF THE METABOLISM STUDY:

The biotransformation of DDVP was studied after oral administration to rats. Male and female rats were dosed with ^{14}C-DDVP (^{14}C-DDVP) at single oral doses, single intravenous dose, and repeated doses (14 daily doses) of unlabeled DDVP followed by administration of a single oral dose of labeled DDVP. Among five radioactive components detected in urine, urea and hippuric acid were identified by mass spectrometric analysis. The amount of hippuric acid was 6.8% to 10.5% (low-dose group) and 4.2% to 5.6% (high-dose group) of the urinary radioactivity. The amount of urea was 19.6% to 33.1% (low-dose group) and 41.5% and 5.1% (high-dose group) of the radioactivity in urine. Hippuric acid and urea were also found in the feces. Three other metabolites were not identified, however, they were assumed to be dehalogenated metabolites.

Enzyme hydrolysis of urine revealed the possible presence of glucuronide conjugates. However, the identity of the hydrolysis products was not elucidated. The proposed metabolic pathway for DDVP is attached to the DER.

The large amount of radioactivity eliminated in the expired air (as carbon dioxide), two identified metabolites (urea and hippuric acid), and the dehalogenated metabolites suggested that the metabolic pathways involve the one-carbon pool biosynthesis mechanism pathway for the natural products and conjugates.

Primary Reviewer: Paul Chin, Ph.D. Paul Chin 3/25/92
Section 2, Toxicology Branch I (H7509C)
Secondary Reviewer: Joycelyn Stewart, Ph.D. 3/25/92
Acting Section Head, Section 2, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Rat (85-1)

Tox Chem No: 328
MRID No: 418399-01
TB Project No: 1-1556

TEST MATERIAL: Dimethyl dichlorovinyl phosphate

SYNONYMS: DDVP

SPONSOR: AMVAC Chemical Corp.

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI

STUDY NO.: HLA 6274-105-1

REPORT TITLE: Supplemnt to: Metabolism of ^{14}C-DDVP in Rats [Preliminary and Definitive Phases] [MRID No. 412287-01]

AUTHORS: Theresa Cheng

REPORT ISSUED: March 28, 1991

CONCLUSIONS:

The biotransformation of DDVP was studied after oral administration to rats. Male and female rats were dosed with ^{14}C-DDVP (^{14}C-DDVP) at single oral doses, single intravenous dose, and repeated doses (14 daily doses) of unlabeled DDVP followed by administration of a single oral dose of labeled DDVP. Among five radioactive components detected in urine, urea and hippuric acid were identified by mass spectrometric analysis. The amount of hippuric acid was 6.8% to 10.5% (low-dose group) and 4.2% to 5.6% (high-dose group) of the urinary radioactivity. The amount of urea was 19.6% to 33.1% (low-dose group) and 41.5% and 5.1% (high-dose group) of the radioactivity in urine. Hippuric acid and urea were also present in the TLC profiles of feces extracts. Three other metabolites were not identified. However, the lack of the characteristic "chlorine clusters" in their mass spectra suggest that the these three components were dehalogenated metabolites.

The large amount of radioactivity eliminated in the expired air (as carbon dioxide), two identified metabolites (urea and

hippuric acid), and the dehalogenated metabolites suggested that the metabolic pathways involve the one-carbon pool biosynthesis mechanism pathway for the natural products and conjugates. The proposed metabolic pathway for DDVP is attached to this DER.

CLASSIFICATION (Core-Grade): Acceptable

The Toxicology Branch I concludes that this study is acceptable, but it does not by itself satisfy the guideline requirements (85-1). In conjunction with a previous rat metabolism study (MRID No. 412287-01, HED DOC. NO. 008132 dated Oct. 19, 1990) these studies satisfy the guideline requirement for metabolism study.

OBJECTIVES

The objective of this supplement was to characterize, quantify, and identify the ^{14}C metabolites of DDVP in excreta samples of rats that received one of the four doses: single i.v. dose (1 mg/kg), single oral dose (1 and 20 mg/kg), and multiple oral doses (1 mg/kg).

MATERIALS:

Samples of urine and feces collected from 0 to 24 hours after the administration, which together represented over 90% of the radioactivity recovered in excreta, were pooled by dose group and sex. All samples were obtained directly from HLA's in-life facility (see HLA Study No. 6274-105 dated Aug. 30, 1989, MRID No. 412287-01; HED Doc. No. 008132 dated Oct. 19, 1990).

Chemical structures for standards and metabolites are shown in Figure 1. **DDVP was labeled with ^{14}C at 1-vinyl position of DDVP.**

PREPARATION OF DOSING SOLUTIONS and TEST ORGANISM:
For details, see ATTACHMENT I

STUDY DESIGN and TREATMENT OF ANIMALS AND ANALYSIS OF EXCRETA & TISSUES:
For details, see ATTACHMENT I

Identification of Metabolites

TLC method

Samples of urine and fecal extracts (0-24 hours) were subjected to chromatographic analyses along with hippuric acid and urea standards.
Solvent systems used were as follows:

Solvent system 1: n-propanol:0.88% ammonium hydroxide (7:3)
Solvent system 2: chloroform:methanol:formic acid: water (70:30:3:3)

High-performance liquid chromatography (HPLC) method

Several HPLC methods were investigated, but none were found to be suitable for sample analysis.

Other methods

The urine and fecal samples were subjected to gas chromatography (GC)-electron capture detector (ECD) method, a glucuronidase hydrolysis, isotope dilution analysis, flash chromatographic method, and mass spectrometric analysis. For details, see ATTACHMENT II.

QUALITY ASSURANCE:

A signed statement of compliance with Good Laboratory Practices and a signed statement of Quality Assurance were included in the submission.

RESULTS:

The excretion of radioactivity in expired air (as carbon dioxide), urine and feces was 39.5% to 57.5%, 10.6% to 16.7%, and 4.4% to 7.1%, respectively.

Identification of Metabolites

Urine

Enzyme hydrolysis of urine samples with glucuronidase revealed the possible presence of glucuronide conjugates (approximately 8% to 19% of the radioactivity in the urine as the ethyl acetate extract). However, the identity of the hydrolysis products was not elucidated.

Five radioactive components from urine sample were analyzed by mass spectrometric analysis. A positive identification was made for urea and hippuric acid only and three other components were not identified. However, the lack of the characteristic "chlorine clusters" in their mass spectra suggest that the these three components were dehalogenated metabolites.

The results of the TLC analyses of urine samples showed that among the six radioactive components detected, only two metabolites had Rf values corresponding to hippuric acid and urea standards. Using the solvent system 2, the amount of radioactivity in the region of hippuric acid was 6.8% to 10.0% for the low-dose group and 4.2% to 5.6% for the high-dose group. Using the same solvent system, the amount of radioactivity in the region of urea was 19.6% to 33.1% for the low-dose group and 41.5% to 51.1% for the high-dose group.

Feces

The results of the TLC analyses of fecal samples showed that among the five radioactive components detected, only two metabolites had Rf values corresponding to hippuric acid and urea standards. Using the solvent system 1, the amount of radioactivity in the region of hippuric acid was 1.7% to 5.5% for the low-dose group and 2.0% to 4.3% for the high-dose group. Using the same solvent system, the amount of radioactivity in the region of urea was 6.7% to 29.7% for the low-dose group and 2.6% to 6.1% for the high-dose group.

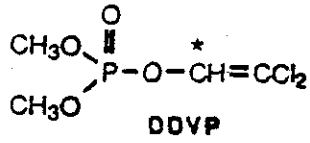
Metabolic Pathways

The major metabolic pathways for DDVP in rats are proposed in Figure 2.

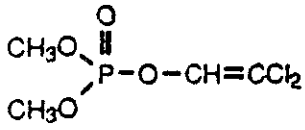
The large amount of radioactivity eliminated in the expired air (as carbon dioxide), two identified metabolites (urea and hippuric acid), and the dehalogenated metabolites suggested that the metabolic pathways involve the one-carbon pool biosynthesis mechanism pathway for the natural products and conjugates.

Figure 1

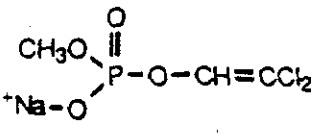
Chemical Structures for Standards



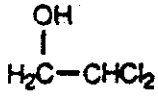
DDVP



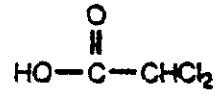
Dichlorovinyl dimethyl phosphate (DDVP)



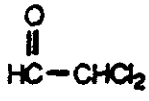
Dichlorovinyl methyl phosphate sodium salt



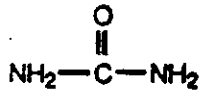
Dichloroethanol



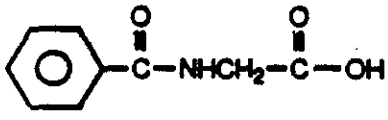
Dichloroacetic acid



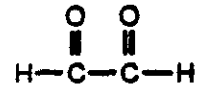
Dichloroacetaldehyde



Urea



Hippuric acid

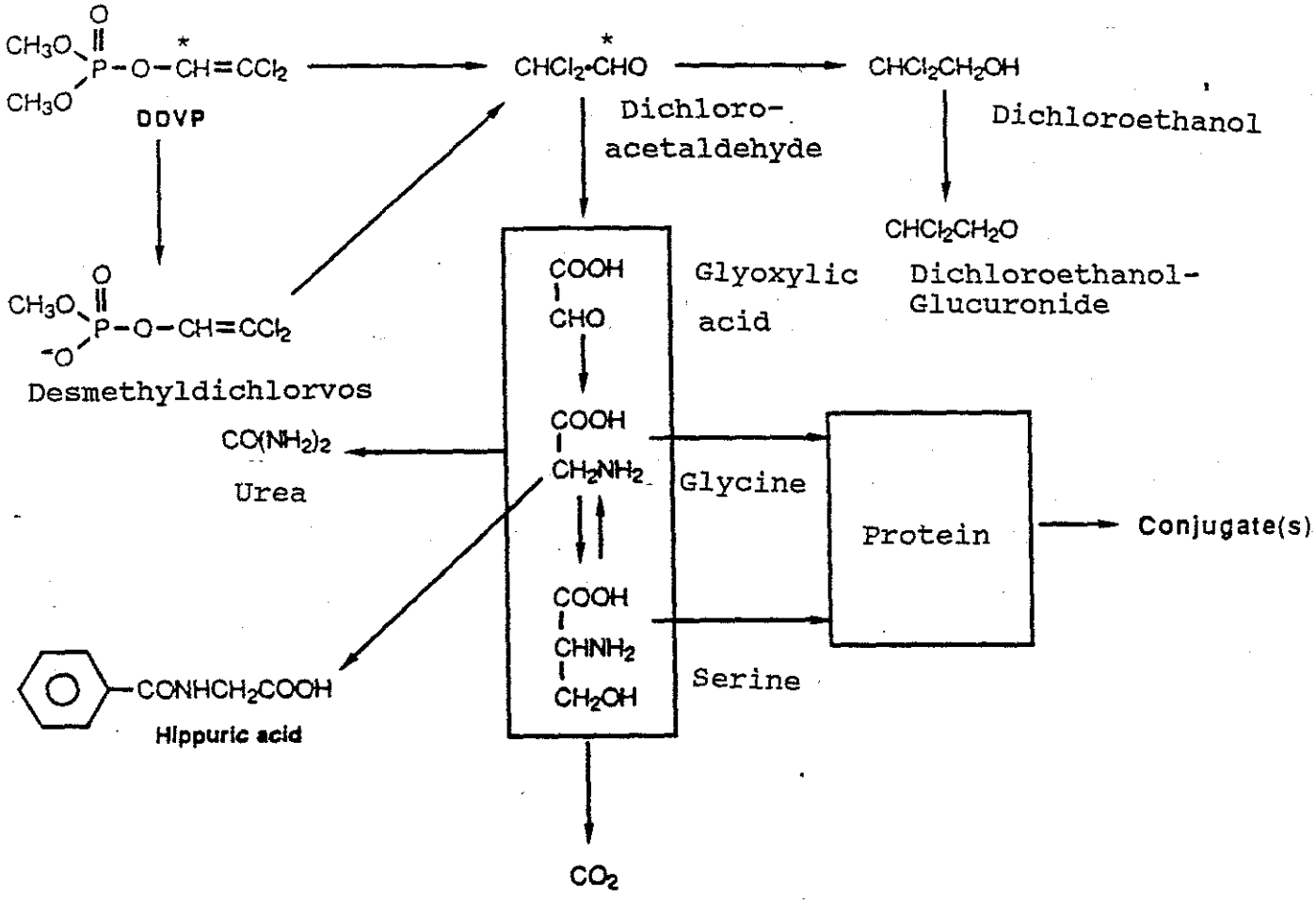


Glyoxal

* Position of ^{14}C

Figure 2

Proposed Metabolic Pathway of ^{14}C-DDVP Administered to Rats



* Position of ^{14}C

Reference: Hutson D. H., Hoadley E. C. (1971) Xenobiotica, Vol 1, No 6, 593-611

ATTACHMENT I (from HED Doc. No. 008132, pp 5-7, dated Oct. 19, 1990, HLA Study No. 6274-105 dated Aug. 30, 1989)

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. [^{14}C]DDVP (lot No. 2534-039) was purified before use in the definitive study by thin-layer chromatography (TLC) and subsequent extraction with methylene chloride. The radiochemical purity of the [^{14}C]DDVP used in dosing solutions was determined to be 100 percent by TLC (with a solvent system of benzene:methanol, 20:1, v/v) and gas chromatography (GC).

2. Male and female Crl:CD(SD)BR rats obtained from Charles River Laboratories (Portage, MI) were used. Animals were 5 to 9 weeks old and weighed between 125 and 200 g at the time of arrival at the performing laboratory. The rats were allowed at least 1 week to acclimate before dosing. Animals were fasted overnight to 4 hours postdose.

3. Dosing solutions were prepared on the day of dosing as described below. Measured amounts of purified [^{14}C]DDVP or unlabeled DDVP in methylene chloride were placed in glass vials, and the organic solvent was evaporated under nitrogen; deionized water was added to a final volume, and the mixtures were sonicated to ensure homogeneity. For the high-dose solution only, unlabeled DDVP was used to dilute [^{14}C]DDVP to the final concentration. The [^{14}C]DDVP in the methylene chloride stock solution was found to be stable over the dosing period when examined by TLC and GC (as described in section 11.A.1 of this DER). The [^{14}C] concentration of the radiolabeled dosing solutions was determined by liquid scintillation counting (LSC) before and after compound administration.

4. Twenty-four rats/sex were used. Animals were randomly assigned to the preliminary-phase study or to one of the five groups in the definitive-phase study (Table 1). The two rats/sex in the preliminary-phase study were given a single oral dose of 1 mg [^{14}C]DDVP/kg. In the definitive-phase study, groups of five rats/sex were administered a single intravenous (iv) dose of 1.0 mg [^{14}C]DDVP/kg; a single oral dose of 1.0 or 20.0 mg [^{14}C]DDVP/kg (low- and high-dose groups, respectively); or a single oral dose of 1.0 mg unlabeled DDVP/kg/day for 15 days followed by a single oral dose of 1.0 mg [^{14}C]DDVP/kg (repeated-dose group). Each rat in both the preliminary-phase and definitive-phase studies received 20 μCi of [^{14}C]DDVP. An additional two rats/sex were given a single oral dose of vehicle only (control group). Oral doses were administered by gavage, and iv-administered test material was injected into the tail vein. The dose given to

each animal was determined by weighing syringes before and after dosing.

Rats were placed in individual metabolism cages after administration of radiolabeled DDVP. Animals were checked twice each day for mortality and moribundity and once daily for other signs of toxicity. Body weights were recorded on the first day of treatment, randomly throughout the study, and on study days 7 and 14 (repeated-dose animals). For animals in the preliminary-phase study, urine and feces were collected separately over ice at 0 to 12 and 12 to 24 hours after dosing and daily thereafter for 7 days; for all other animals, urine and feces were collected over ice at 0 to 6, 6 to 12, and 12 to 24 hours postdosing and at 24-hour intervals thereafter for 7 days after compound administration. Expired air (i.e., $[^{14}C]CO_2$) was trapped in a solution of ethanolamine:ethoxyethanol (1:3) at the same time intervals described above. Activated charcoal was used to trap radiolabeled organic volatiles exhaled by animals in the preliminary-phase study only. Cages were rinsed with 1 percent trisodium phosphate at the end of the 7-day collection periods. Animals were sacrificed at 24 hours after dosing (controls) or at 7 days postdosing (all test animals), and the following tissues were collected, weighed, and radioassayed: blood, bone (femur), brain, fat, ovaries/testes, heart, pancreas, liver, kidneys, lungs, muscle (thigh), spleen, uterus, and residual carcass.

5. Aliquots of urine, cage washes/wipes, and CO_2 traps were analyzed directly for $[^{14}C]$ content by LSC. Whole blood samples were combusted and then counted, and feces and all tissues (including the carcasses) were homogenized, combusted, and radioassayed. External standards and an instrument-stored quench curve were used to determine counting efficiencies and to minimize color quenching.

B. Protocol: A protocol for this study is not included in this DER.

12. REPORTED RESULTS:

A. All high-dose rats exhibited tremors and salivation; one female in this group died 2.5 hours after dosing. Several animals in the iv- and repeated-dose groups had dark urine, and one repeated-dose male reportedly consumed no food on days 16 through 20.

ATTACHMENT II (from Study Report HLA 6274-105-1, pp 12-13, dated Aug. 30, 1989)

Gas Chromatography (GC) Method. A GC-electron capture detector (ECD) method was also investigated using the experimental conditions listed below:

Instrument:	Hewlett Packard Gas Chromatograph Model 5730A with ECD
Column:	20M 3% Carbowax
Injection Port Temperature:	250°C
Temperature Program #1:	80°C for 4 minutes 80°C to 150°C at 4°C/minute 150°C for 4 minutes

Glucuronidase Hydrolysis

Selected urine samples were treated with ß-glucuronidase (Sigma Type B-1, from bovine liver, Lot Nos. 117F-7256, 108C-7180, and 32F-7285). The enzyme was added to the urine samples (1 to 50 mL), the sample was suspended in buffer (0.1M sodium acetate, pH 4.6), and the mixture was incubated overnight at 37°C in a shaking water bath. A urine sample without added enzyme was treated in the same manner to serve as a control. The hydrolysates were extracted with ethyl acetate or methanol and subjected to GC or TLC analysis.

Isotope Dilution Analysis

Known amounts of authentic standards (hippuric acid and urea), ranging from 3 to 4 g, were added to lyophilized urine samples (ranging from 4 to 40 mL) separately. The mixture was dissolved in boiling ethanol, then filtered. The filtrate was allowed to recrystallize at room temperature. The supernatant was drawn off and stored in the freezer. The crystals were dried under a stream of nitrogen, and duplicate aliquots were weighed and counted to determine the specific activity. The remaining crystals were then redissolved in boiling ethanol, and the recrystallization steps were repeated. The process was repeated for hippuric acid and urea until the specific activities of three consecutive recrystallizations were within 10% or until there was no longer enough crystals to work with.

Isolation of Metabolites

Flash Chromatographic Method. A glass column (2 ft x 2 cm id) was packed using the following method: A small plug of glass wool was placed in the bottom of the column, followed by a layer of sea sand. Silica gel was then poured over the sand to a level of approximately 6 inches. The appropriate solvent was then carefully poured over the silica and allowed to percolate through the column. After the solvent started to drip through the column, nitrogen pressure was applied to force the solvent through, along with air bubbles, the silica gel. After all the air bubbles had passed through the column, more solvent was applied to the top of column and another layer of sea sand was poured on top of the silica. The solvent was forced through the column by nitrogen pressure to approximately 1 in. from the top of the column. The selected sample (approximated 1.0 to 3 mL of the extract) was then applied to the top of the column using a pipette, and the sides were rinsed with the initial solvent. Nitrogen pressure was again applied to push the

Several solvent systems were investigated. The following solvent systems were used for large-scale preparations:

Solvent System 3:

5:1	n-Propanol:hexane
90:10	n-Propanol:0.88% ammonium hydroxide
60:40	n-Propanol:0.88% ammonium hydroxide

Solvent System 4:

5:1	n-Propanol:hexane
95:5	n-Propanol:0.88% ammonium hydroxide
90:10	n-Propanol:0.88% ammonium hydroxide
85:15	n-Propanol:0.88% ammonium hydroxide
80:20	n-Propanol:0.88% ammonium hydroxide
75:25	n-Propanol:0.88% ammonium hydroxide
70:30	n-Propanol:0.88% ammonium hydroxide
60:40	n-Propanol:0.88% ammonium hydroxide

Preparative TLC Method. Each fraction collected from the flash chromatography column was concentrated and applied to a preparative TLC plate (Silica-Gel 60, 20 cm x 20 cm, 0.5 mm). The plates were developed using TLC Solvent System 1 (n-propanol:0.88% ammonium hydroxide 7:3). The radioactivity bands were detected using a TLC linear analyzer. Bands with similar R_f values were marked, scraped, combined, and the radioactivity was extracted with methanol.

Mass Spectrometric Analysis. Mass spectrometric analysis was done via fast atom bombardment (FAB) at the Department of Biochemistry, University of Wisconsin (Madison, Wisconsin).

RESULTS AND DISCUSSION

Total Radioactivity Concentrations in Excreta

The ^{14}C concentrations in the pooled excreta samples that were used for metabolite characterization are summarized in Table 1. For urine samples, the radioactivity concentrations ranged from 0.917 to 1.84 ppm ^{14}C-DDVP equivalent for the low-dose groups (Groups 1 to 3) and were 26.9 and 31.3 ppm for male and female rats, respectively, in the high-dose group. The radioactivity concentrations in the composite fecal samples were considerably lower than in the urine samples. For the low-dose groups, radioactive concentrations ranged from 0.106 to 0.269 ppm. For the high-dose group, the concentration in feces ranged from 3.80 to 5.82 ppm.

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Chemical:	**Dichlorvos**
PC Code:	**084001**
HED File Code	**13000 Tox Reviews**
Memo Date:	**04/17/1992**
File ID:	**TX009444**
Accession Number:	**412-01-0083**

HED Records Reference Center
01/11/2001